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Analysis of curative effects of human gamma globulin on bacterial pneumonia in pediatric patients

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Abstract: The main aim of this study was to investigate the effects of human gamma globulin (HGG) on inflammation targets in children. A total of 80 children were randomly divided into observation and control group with 40 cases in each group. The control group was given comprehensive treatment while the observation group was treated with HGG. The time of disappearance of clinical signs and symptoms, time of improvement of pulmonary iconography, inflammatory indices, time and degree of improvement of lung function and adverse reactions were observed. The total effective rate in the observation group was 97.5% and significantly higher than control group (77.5%). The time of fever clearance, imaging improvement as well as cough and pulmonary rales disappearance in the observation group was shorter than control group. After treatment, the levels of inflammatory indicators such as erythrocyte sedimentation rate (ESR) and C-reaction protein (CRP) in the observation group were lower than control group. No obvious abnormalities of urea nitrogen, creatinine, serum alanine amino transferase (ALT) and aspartate amino transferase (AST) were found in the two groups. Overall, HGG effectively shortened the course of RMPP, improved the cure rate, reduced the inflammatory reaction and promoted the recovery of lung function without obvious adverse reaction.

Keywords: Refractory *Mycoplasma pneumoniae* pneumonia, human gamma globulin, inflammatory indexes.

INTRODUCTION

Mycoplasma pneumoniae pneumonia (MPP) is a common disease in pediatrics mainly resulting from mycoplasma pneumoniae infection and manifested with fever, cough, headache, sore throat, skin rash, anemia, hematuria and pulmonary rales that causes a serious impact on the health, life and learning of child patients. In the past, Mycoplasma pneumoniae pneumonia was regarded as a self-limited disease and conditions could be alleviated without special treatment or with significant efficacy of macrolide antibiotics (Bebéar and Pereyre, 2005). In recent years, the conventional treatment of macrolide antibiotics is unsatisfactory and the number of RMPP sufferers has shown an upward trend year by year with complex pathogenesis and disease condition. The long-term use of macrolide antibiotics is easy to cause drug resistance particularity in children, posing a huge challenge to clinical treatment of RMPP (Sone *et al*., 2017). With the continuously deepened researches on RMPP in China, new drugs are emerging in an endless stream and are playing a great role in the treatment of refractory Mycoplasma pneumoniae pneumonia. Gamma globulin, a "protein produced by β cells of the human lymphoid system" is the main effect or of the human immune system (Principi and Esposito, 2002). Immunoglobulin G (IgG) is the main component of gamma globulin and includes such subclasses as IgG1, IgG2, IgG3 and IgG4. It is the most persistent and important antibody in the primary immune response. Most antibodies against bacteria, viruses and toxins belong to the class of IgG, which spread to extra vascular space more easily than other immunoglobulins and play the effects of resisting infection as well as regulating and neutralizing bacteriotoxin (Esposito *et al*., 2002; Tamura *et al*., 2005). IgG is the only immunoglobulin that passes through the placenta. It starts to synthesize on the third month of baby birth and extends to adult level in the period of 8-10 years old, playing an important role in natural passive immunity. The gamma globulin used for intravenous injection is separated from plasma from a large number of donors and it, with abundant autoantibodies such as anti-human tumor necrosis factor-α (TNF-α), interleukin-1(IL-1) and interleukin-6 (IL-6), can directly neutralize pathogens to stimulate cytokines produced by the body, thereby playing an immediate effect of anti-inflammation. In our hospital, human gamma globulin (HGG) has recently been used in the clinical treatment of children with refractory Mycoplasma pneumoniae pneumonia and reached satisfactory results. Combined with clinical practices, the effect of HGG on the curative effect, symptoms, signs and inflammatory indexes in children with RMPP is discussed in this study.

MATERIALS AND METHODS

Patient's selection

A total of 80 children with RMPP treated in "Zhongda Hospital of South-east University, Nanjing, China" from February 2016 to February 2017 were selected as the objects consisting of 45 boys and 35 girls. Among them, 32 cases were up to 5 years old, 48 cases older than 5

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years, with an average age of (7.2±1.4) years and a disease course was 2-12days, (5.2±1.5)days on average. All patients had fever, cough and pulmonary rales including medium bubbling rales in 60 cases and wheeze in 10 cases. The chest radiography (CT) showed that there were 50 cases with bilateral or unilateral atelectasis and lung consolidation; 18 cases with moderate or large pleural effusion; 8 cases with necrotizing pneumonia and 6 cases with bilateral diffuse interstitial disease.

Inclusion criteria

Patient was diagnosed with the clinical standard of mycoplasma pneumonia and in line with any one from following three items for diagnosis of RMPP:

1. Patient treated by macrolide for 1 week or more but with unimproved symptoms accompanied by persistent fever, severe cough and prolonged duration;
2. The imaging showed bilateral or unilateral atelectasis, pulmonary consolidation, lung abscess and moderate or large pleural effusion;
3. Patient with other serious complications besides severe pneumonia lesions.
4. The enrolled 80 patients were randomly divided by random number table into observation group and control group, with 40 cases in each group.

Treatment

The control group was administered standardized comprehensive therapy including condition-based oxygen therapy, defervescence, stopping coughing and dissipating phlegm, bronchospasm alleviation by inhalation and organotherapeutic medicament for organ damages. Patients were injected with azithromycin ($10mg \cdot kg^{-1}d^{-1}$) for continuous 5 days following a discontinuation of 2 days as course-1 of treatment and they were treated for a total of 2-3 courses. After the improvement of condition, some children were treated with oral azithromycin ($10mg \cdot kg^{-1} \cdot d^{-1}$) for a total of 1-2 courses with constant 3 days plus a discontinuation of 4 days as 1 course. Glucocorticoids therapy and fibro bronchoscope treatment was adopted when necessary and patients with identified bacterial infection were given appropriate antibiotics as well as other symptomatic therapy.

Patients in the observation group were treated with gamma globulin ($400mg \cdot kg^{-1} \cdot d^{-1}$) injection for 5 days in addition to the treatment in the control group on the first day of admission, during which the presence of adverse reactions such as allergy was observed.

Observation index

The disappearance time of symptoms like fever, cough, pulmonary rales and the time of improvement of pulmonary iconography as well as inflammatory markers erythrocyte sedimentation rate (ESR) and C-reaction protein (CRP) were observed in the two groups. Venous blood was collected from patients in the early morning on the tenth day of treatment for detection followed by comparison of improvement of lung function which was assessed respectively on the first, third and fifth day. Children over the age of 5 (a total of 48 cases), 24 cases in the observation group and 24 cases in the control group were examined on pulmonary ventilation function followed by a comparison of changes in forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC) and maximal mid expiratory flow (MMEF). In children under the age of 5 (a total of 32 cases),16 cases in the observation group with 16 cases in the control group, tidal breathing parameters was compared and tidal volume (VT), respiratory rate (RR), inspiratory-to-expiratory ratio (tI/tE) as well as time to peak ratio (tPTEF/tE) were monitored. Meanwhile the presence of allergy was observed in children and venous blood was collected to detect the liver and kidney function in the morning of the tenth day with the observation on adverse reaction.

Evaluation of curative effect

(a) ***Markedly effective****:* The symptoms disappeared and the lung imaging showed a recovery of over 50% in 10 days of treatment.

(b) ***Effective****:* The above symptoms were improved and the lung imaging showed a certain recovery less than 50% in 10 days of treatment.

(c) ***Invalid****:* The above symptoms were unimproved and the lung imaging showed no recovery in 10 days of treatment.

Total effective rate= (Markedly effective+ Effective)/ cases in each group*100%.

Ethical approval

The study was approved by the institutional ethical review board of Zhongda Hospital of Southeast University, Nanjing, China. The reference number was 675/IRB-ZHC/2016.

STATISTICAL ANALYSIS

SPSS 22 software was used for statistical analysis. The measurement data was expressed by "mean± standard deviation" and assessed by "T test". The Count data was tested by "χ^2 test" with $P<0.05$ suggested that there was statistically significant difference.

RESULTS

Comparison of effective treatment rate

The total effective treatment rate in the observation group was 97.5%, significantly higher than that in the control group (77.5%) and the difference was statistically significant ($P<0.05$) (table 1).

Table 1: Comparison of effective treatment rate between the two groups

Group	Case	Markedly Effective	Effective	Invalid	Total Effective Rate (%)
Observ: Group	40	29	10	1	97.5
Control Group	40	20	11	9	77.5
X^2	---	---	---	---	6.146
P	---	---	---	---	<0.05

Table 2: symptoms and signs disappearance time and imaging improvement time in the two groups ($\bar{x} \pm s$, d)

Group	Case	Fever Clearance Time	Cough Alleviation Time	Pulmonary Rales Disappearance Time	Lung Imaging Improvement Time
Observ: Group	40	6.4±1.8	8.4±2.1	10.5±2.5	7.4±2.8
Control Group	40	11.9±2.5	11.2±3.1	12.9±3.9	11.2±2.6
t	_	7.054	5.166	5.413	5.986
P	_	<0.05	<0.05	<0.05	<0.05

Table 3: Inflammatory markers in the two groups after treatment ($\bar{x} \pm s$)

Group	Case	Esr (Mm/H)	Crp (Mg/L)
Observ: Group	40	16.5±3.2	16.7±4.2
Control Group	40	21.7±3.5	25.1±6.3
t	---	5.733	6.129
P	---	<0.05	<0.05

Table 4: Lung function in children over the age of 5 in the two groups after treatment ($\bar{x} \pm s$) (n=24)

GP	On The First Day			On The Third Day			On The Fifth Day		
	FEV1	FVC	MMEF	FEV1	FVC	MMEF	FEV1	FVC	MMEF
O/G	43.1±9	46.7±8	32.1±7	47.1±9	55.1±7	61.6±5	66.3±6	60.9±5	66.9±8
C/G	43.2±9.	45.9±7.6	30.6±6.8	46.5±8.9	49.6±7.2	55.3±5.3	51.7±6.1	56.4±6.1	54.2±6.6
T	0.104	0.321	1.526	0.651	4.893	4.902	6.005	4.248	6.093
P	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Where, GP, O/G & C/G indicates Group, Observation Group and Control Group respectively.

Comparison of symptoms and signs disappearance time and imaging improvement time

The time of fever clearance, imaging improvement as well as cough alleviation and pulmonary rales disappearance in the observation group were significantly shorter than those in the control group (P<0.05) (table 2).

Comparison of inflammatory indexes after treatment

After treatment, the levels of such inflammatory indicators as ESR and CRP in the observation group were significantly lower than those in the control group and the difference was statistically significant (P<0.05) (table 3).

Comparison of lung function after treatment

While comparing routine pulmonary ventilation function in children over 5 years old, there was no significant difference between the two groups in forced expiratory volume in one second (FEV1), forced vital capacity (FVC) and maximal midexpiratory flow (MMEF) on the first day of treatment (P>0.05). On the third day, there was no significant difference in FEV1 (P>0.05), while FVC and MMEF improved significantly in the observation group compared with the control group (P< 0.05). On the fifth day, FEV1, FVC and MMEF were significantly improved in the observation group compared with the control group (P<0.05) (table 4).

Comparison of lung function after treatment

In children under the age of 5, there was no significant difference between the two groups in tidal volume (VT), respiratory rate (RR), inspiratory-to-expiratory ratio (tI/tE) and time to peak ratio (tPTEF/tE) on the first day (P>0.05) and in VT on the third day (P>0.05), while on the fifth day, VT was significantly improved in the observation group compared with the control group (P<0.05). There was no significant difference between the two groups in RR (P>0.05) but RR on the third day in the two groups was significantly improved compared with that on the first day in the same group (P<0.05). On the third and fifth day, tI/tE and tPTEF/tE were significantly improved in the observation group compared with the control group, (P<0.05) (table 5).

Adverse reactions

No obvious abnormalities of urea nitrogen, creatinine, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were found in the two groups before and after treatment, and there was 1 case with skin rash in each group, which was subsided in one day after oral loratadine with no serious adverse reactions such as anaphylactic shock.

DISCUSSION

Mycoplasma pneumoniae pneumonia is a common frequently-occurring disease mainly caused by Mycoplasma pneumoniae (MP) infection and mainly pathologically manifested as interstitial pneumonia and potential bronchopneumonia, and it, known as primary atypical pneumonia, has mild clinical symptoms of fever, cough and headache with a certain lack of specificity, thus easy to be neglected by parents of child patients (Vervloet *et al*., 2007; Gaillat *et al*., 2005). Mycoplasma pneumonia in children has the characteristics of acute disease, rapidly changing condition as well as high recurrence rate and will lead to serious consequences if there is no timely or improper treatment. In clinical trials it is mainly treated by drugs in which macrolide antibiotics is used more commonly with remarkable effects. But the symptoms of Mycoplasma pneumoniae pneumonia in some patients fail to be improved with the condition in progress after proper treatment of macrolide antibiotics and even induce respiratory failure, respiratory distress as well as septic shock when serious to directly endanger patient safety, which is defined by most experts as refractory Mycoplasma pneumoniae pneumonia (RMPP) (Zhang *et al*., 2016; Narita, 2016). The pathogenesis and mechanism of RMPP is rather complex and may be closely related to the pathogenesis of Mycoplasma pneumoniae pneumonia, especially associated with immune factors, mixed infection as well as drug resistance and prone to misdiagnosis as well as missed diagnosis. Due to the poor efficacy of conventional antibiotics in the treatment of children with RMPP, it is required to explore a safe and effective treatment plan so as to relieve patient discomfort and improve cure rate of the disease.

In recent years, immunological factors have been considered to play an important role in the occurrence and development of RMPP. The degree of damage caused by MP infection is positively correlated with host immune response after the infection and not entirely direct or indirect microbial injury (Tamura *et al*., 2008; Nebrera and Ramirez, 2015), which provides a theoretical basis for the treatment with gamma globulin in this study. RMPP has features of acute onset and fast progress with symptoms of high fever and severe coughing and is likely to induce serious lung damage with obvious extra-pulmonary complication in the short term (Holt *et al*., 2015). The extra pulmonary complications of RMPP are most common in the cardiovascular system, the liver as well as the digestive system and some in the nervous system. In terms of treatment, the anti-MP therapy is still emphasized first because MP has no cell wall and makes no response to P- lactams, macrolides can inhibit MP by interfering and inhibiting protein synthesis and leads to moderately fewer adverse effects, thus being the first choice for anti-MP treatment. Azithromycin sequential therapy is adopted in clinical practices because azithromycin can largely accumulate in tissues with short treatment course and good tolerance. In children with erythromycin-emia, erythromycin can be used for treatment because of its special drug metabolism and high concentration in blood. However, RMPP has rapid progress and easily leads to serious complications such as necrotizing pneumonia, bronchitis obliterans, organizing pneumonia, serious decline in lung function as well as multi organ failure, thus making the simple application of macrolide drugs achieve poor efficacy, so the purpose of this study is to further strengthen the treatment of RMPP and reduce the incidence of complications.

In this study, we focused on the immune response induced by MP and the results showed that MP infection was followed by the disorders of innate immune system, cell immune system and humoral immune system (Sánchez-Ramón *et al*., 2016). A study on peripheral blood and Broncho alveolar lavage fluid (BALF) following MP infection revealed that there was imbalance of TH1/TH2 and significant increase of IL-4 and INF7 in many cytokines in acute stage of MP, resulted in immune disorder and decreased immunity. MP infection can cause a reduction in the proliferation and differentiation of B lymphocyte, thus leading to the resistance to humoral immunity related antibodies, lower immune function as well as reduced abilities of anti-infection and anti-toxin. Gamma globulin contains a variety of antibodies extracted from plasma, mainly IgG and also a small amount of IgA and IgM. It plays an important role in severe pneumonia, sepsis, mycoplasma infection and other infectious diseases. Intravenous injection of gamma globulin enables to rapidly increase IgG concentration in blood, enhance immunity and neutralize pathogens (Tamura *et al.,* 2008). It also plays the effect of immune regulation in many ways, regulates function of B lymphocyte, activates the activity of complement system and thus promotes the recovery of body's immune function. In RMPP treatment with intravenous injection of gamma globulin the time of symptoms like fever and cough alleviation as well as rale absorption was significantly shorter in the observation group than in the control group and there was no incidence of adverse reaction. RMPP patients with neurological complications often had encephalitis and few of them suffered from Green Barre syndrome, peripheral neuritis or polyneuritis

Table 5: Lung function in children under the age of 5 in the two groups after treatment ($\bar{x} \pm s$)

GP	Case	On The First Day				On The Third Day				On The Fifth Day			
		VT (Ml/Kg)	RR (Time/Min)	Tl/Te	Tptef/Te (%)	VT (Ml/Kg)	RR (Time/Min)	Tl/Te	Tptef/Te (%)	VT (Ml/Kg)	RR (Time/Min)	Tl/Te	Tptef/Te (%)
O/G	16	4.4±1.2	45.4±7.6	0.46±0.15	16.8±3.2	6.6±0.5	31.8±3.4	0.69±0.05	23.7±2.6	7.8±0.9	28.6±2.6	0.77±0.06	28.1±1.8
C/G	16	4.4±1.3	44.6±8.3	0.48±0.16	17.2±3.5	5.8±1.1	32.2±4.6	0.57±0.08	20.1±2.9	5.9±1.4	30.6±2.8	0.66±0.05	24.7±1.5
T		0.105	0.400	0.982	1.095	1.456	0.348	3.069	4.099	7.007	0.904	5.483	5.022
P		>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

of moderately serious condition. Gamma globulin had good therapeutic effect in treatment of MP associated encephalopathy, especially 5 days after the disease, which might was related to immune-mediated encephalitis.

The results of this research showed that in the observation group, the total effective rate was significantly higher and the time of fever clearance, imaging improvement as well as cough and pulmonary rales disappearance were significantly shorter by contrast. This suggested that the treatment of gamma globulin combined with azithromycin for RMPP promoted improvements in clinical symptoms, signs and imaging. The study also showed that the levels of inflammatory markers like ESR and CRP were decreased more obviously in the observation group than in the control group which suggested that gamma globulin had good anti-inflammatory effect. CRP is a nonspecific inflammatory marker. It will rapidly and significantly increase in the cases of acute myocardial infarction, infection, inflammation, trauma, surgery and tumor infiltration. The content of CRP in serum is extremely low in normal people, but can increase dramatically in a short period of time with tissue injury inflammation and infection, playing an important role in protection of innate immune. It will gradually decrease to a normal level when the condition is improved, positively correlated with the severity of MPP and also associated with excessive immunity of RMPP. Studies have shown that CRP can be used as a sensitive indicator for early judgment or prediction of the severity of RMPP and the presence of bacterial infection. In clinical work, the lack of knowledge about early RMPP resulted in improper treatments and aggravated disease progressing to necrotizing pneumonia or severe pneumonia, leaving sequela and even threatening patient life. The decline of CRP was used as a reference index for the improvement of the RMPP inflammatory response and helped to determine whether the treatment was effective.

RMPP caused serious damages to respiratory mucous and there was presence of mucosa hyperemia and edema, ulcer, sputum obstruction, stenosis, granulation hyperplasia and even lumen occlusion by branch of iberoscope. The function of lung ventilation was also prone to decline with severe injury, which involved the adhesion of P1 protein and immune injury. The gamma globulin can regulate the immune function, reduce the injury of lung mucosa and accelerate the recovery of lung function. Studies have shown that (Ahmed and Wishah, 2016). Children with RMPP were more likely to have severer mixed ventilation disturbance, mainly mild and moderate restrictive ventilation disturbance as well as severe obstructive ventilation disturbance, and lung function could gradually return to normal after appropriate treatment. In this study, the children over 5 years old were examined on pulmonary ventilation. The results showed that the levels of FEV1, FVC and MMEF

were significantly reduced in both groups, suggestive of mild and moderate restrictive ventilation disturbance or severe obstructive ventilation disturbance; According to the results FVC and MMEF were markedly improved in the observation group compared to the control group on the third day of treatment and on the fifth day, FEV1, FVC and MMEF were improved, suggestive of significantly improved pulmonary ventilation. The detection of tidal breathing parameters including VT, RR, tI/tE, tPTEF/tE was conducted in children under 5 years old and the results showed that VT was improved significantly in the observation group. The pulmonary ventilation function improved significantly after the treatment with gamma globulin, suggesting that gamma globulin can promote the recovery of lung function by regulating immune function.

CONCLUSION

HGG effectively shortened duration of refractory Mycoplasma pneumoniae pneumonia, improved treatment efficiency and reduced inflammatory response of safety and reliability.

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